

The composition of reagents R<sub>1</sub> and R<sub>2</sub> was as follows:

Reagent R <sub>1</sub>		
1. Substrate	235 ul	
2. Antibody (1:100)	25 ul	
3. Complement (1:2)	40 ul	
4. Surfactant (1%)	40 ul	
5. TRIS Buffer	35 ul	
Reagents R <sub>2</sub>		
1. Liposome (1:20)	15 ul	
2. TRIS Buffer	30 ul	
3. Surfactant (1%)	5 ul	

The liposomes were made as described in Example I, except that a phenobarbital conjugate was used. All of the other stock solution preparations were as described in Example I, except that anti-phenobarbital rabbit antiserum from Cappel Laboratories, supra, was used.

The determinations were performed on an RA-1000 analytical system according to the manufacturer's protocol (Technicon Instruments Corporation, Tarrytown, NY).

The same comparisons were made as in Example I to determine the effect of various surfactants. The results are shown in Table II.

TABLE II

EFFECT OF SURFACTANTS ON PHENOBARBITAL LIPOSOME		
Surfactant	AB <sup>+</sup> /AB <sup>-</sup>	S <sup>+</sup> /S <sup>-</sup>
TRIS buffer	6.8	1
Igepal CO-630	—	6.8
Igepal CO-710	—	6.8
Igepal CO-880	6.5	1
Triton X-100	—	6.8
Triton X-102	—	6.8
Triton X-305	6.5	1
Brij-35	6.5	1
Pluronic L-43	6.8	1
Pluronic P-84	6.8	1
Pluronic P-85	6.8	1
Pluronic P-104	6.8	1
Pluronic P-105	6.8	1
Tetronic 504	6.4	1
Tetronic 704	6.4	1
Tetronic 904	6.4	1
Tetronic 1104	6.4	1
Tetronic 1304	6.4	1
Tetronic 1504	6.4	1

### Conclusions

Triton and Igepal surfactants with 10-20 EtO monomer units were found to lyse liposomes (S<sup>+</sup>/S<sup>-</sup> = 6.5-6.8) and, thus, effectively prevented any determination based on immunolysis. However, Brij-35, Triton X-305 and Igepal CO-880 did not lyse the liposomes. Likewise, Tetronic surfactants with 30 or more EtO monomers and Pluronic surfactants having 11 to 73 EtO monomers do not lyse the liposomes (S<sup>+</sup>/S<sup>-</sup> = 1).

### EXAMPLE III

#### Phenobarbital Immunoassay Using Pluronic 105 Surfactant

The experiments reported by this example demonstrate the use of an immunoassay composition in accordance with the invention for determination of phenobarbital in an automated clinical analyzer.

Reagents R<sub>1</sub> and R<sub>2</sub> used here were exactly as those described in Example II, using Pluronic P-105 as surfactant, and the experiments were performed on an RA-

1000 system according to the protocol provided by the manufacturer (Technicon, supra).

Dose/response relationships were observed using phenobarbital liposomes with and without surfactant. Emit controls (Syva Company, Palo Alto, CA) having phenobarbital concentrations of 0, 5, 10, 20, 40 and 80 ug/ml were used as "ligand-containing sample" to generate data demonstrating this dose/response relationship. The effect of the presence and absence of Pluronic P-105 surfactant on lysis of liposome is given in Table III.

TABLE III

EFFECT OF PLURONIC 105 ON PHENOBARBITAL DETERMINATION		
Phenobarbital (ug/ml)	% Lysis	
	Without	With
0	100	100
5	85	84
10	69	66
20	52	50
40	19	19
80	6	6

As can be seen from these data, the Pluronic 105 surfactant had no effect on the immunoassay reactions or resulting accuracy of the reported ligand concentration at any point over the entire range covered.

Then, a comparison study in which phenobarbital was determined in human serum samples was carried out in the presence and absence of Pluronic 105 surfactant on the RA-1000 system as described above. The correlation coefficient was found to be 0.986 with a slope of 0.97.

Thus, it has been demonstrated that the present invention provides an immunoassay reagent and method which is dose-responsive and provides results which correlate well with the same method in the absence of surfactant.

### EXAMPLE IV

#### Phenobarbital Immunoassay Using Tetronic 704 Surfactant

The experiments reported by this example demonstrate the use of an immunoassay composition in accordance with the invention for determination of phenobarbital in an automated clinical analyzer.

Reagents R<sub>1</sub> and R<sub>2</sub> used here were exactly as those described in Example II, using Tetronic 704 as surfactant, and the experiments were performed on an RA-1000 system according to the protocol provided by the manufacturer (Technicon, supra).

Dose/response relationships were observed using phenobarbital liposomes with and without surfactant. Emit controls (Syva Company, Palo Alto, CA) having phenobarbital concentrations of 0, 5, 10, 20, 40 and 80 ug/ml were used as "ligand-containing sample" to generate data demonstrating this dose/response relationship. The effect of the presence and absence of Tetronic 704 surfactant on lysis of liposome is given in Table IV.

TABLE IV

EFFECT OF TETRONIC 704 ON PHENOBARBITAL DETERMINATION		
Phenobarbital (ug/ml)	% Lysis	
	Without	With
0	100	100